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SHORT COMMUNICATION

Seroprevalence of Rickettsia spp. infection among tick-bitten patients and blood donors in Sweden

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Abstract

Serum samples from 236 Swedish patients with symptoms of infectious disease appearing after a tick bite were analysed for the presence of antibodies to Rickettsia helvetica, the only rickettsial species so far isolated from ticks in Sweden. Of these subjects, 137 had tested seropositive for Borrelia burgdorferi. For control purposes, sera from 161 healthy blood donors were examined. A total of 10/397 samples (2.6%) showed IgG-antibodies to R. helvetica at or above a titre of 1/80 as cut-off. 6/137 (4.4%) belonged to the Borrelia positive group, 3/99 (3.0%) to the tick-bitten but Borrelia negative group and 1/161 (0.6%) to the control group. The difference between the tick-exposed groups and the control group was significant in Pearson's 2-sided χ^2 test. In 1 serum sample the presence of antibodies to R. helvetica was further confirmed by Western immunoblot. The study shows that infection with Rickettsia spp. as well as coinfection with Lyme borreliosis needs to be considered in the diagnosis of tick-transmitted infections in Sweden. Owing to a known occurrence of immunological cross-reactivites, however, the results must be cautiously interpreted with regard to species of Rickettsia involved.

Introduction

Several emerging tick-borne spotted fever rickettsioses have been reported in Europe during the last decade, including Rickettsia helvetica [1]. The estimated prevalence of R. helvetica in the Ixodes ricinus tick varies between 2.5% and 22% in different European countries, and R. helvetica is to date the only rickettsia isolated in Sweden [2,3]. Ticks are also vectors of other agents such as Borrelia burgdorferi, Anaplasma phagocytophilum, tick-borne encephalitis virus and, in rare cases, Babesia, and possible coinfections have been proven [4]. In Sweden I. ricinus is the only reported tick associated with R. helvetica. In Japan, however, a rickettsia identical or closely related to R. helvetica has been isolated from I. ovatus, I. persulcatus and I. monospinosus ticks [5].

The pathogenic role of R. helvetica remains a matter of controversy but has been implicated in fatal perimyocarditis, sarcoidosis and valvular disease [6–8]. Serological evidence of infection associated with fever, myalgia and no cutaneous rash has been reported from patients in France, Italy and Thailand and in tick-bitten patients from Switzerland [2,9–12]. In a study from Laos, seroconversion to R. helvetica was described as the cause of fever in 2.6% of adults admitted to hospital [13]. In a Swedish patient, R. helvetica was recently also reported to cause septicaemic eruptive febrile illness and long-lasting myasthenia (unpublished observations). Apart from a serosurvey in France, Denmark and Italy, the prevalence of antibodies to R. helvetica in other areas in Europe is to date unknown [2,10,14]. This study is a first attempt to screen for the infection in Sweden. Sera from Swedish patients that were tick-bitten, half of them also seropositive for B. burgdorferi, and a group of healthy blood donors were screened for rickettsial antibodies through an indirect microimmunofluorescence assay (MIF), using R. helvetica as antigen. One serum sample was further analysed by Western blot.

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Materials and methods

The study was approved by the Ethics Committee, Uppsala University.

Study population

A total of 236 Swedish patients seeking medical attention with various symptoms after a previous tick bite were analysed for the presence of rickettsial antibodies. The patients had been exposed to ticks and in most cases tick-bitten during the last month but with a range from 3 weeks to 2 y. Common symptoms were headache, a feeling of sickness, and myalgias. The median age of the patients was 51 y, range 4–93 y. All samples were collected between 2002 and 2006 from the central areas of Sweden and submitted to the Department of Clinical Microbiology, Falu Central Hospital. Anti-B. burgdorferi IgG and IgM antibodies were measured by an indirect enzyme-linked immunosorbent assay (ELISA) using B. burgdorferi as test antigen (DAKO, Glostrup, Denmark). 104 of the 137 samples positive for B. burgdorferi were positive for IgG only and 33 were positive for both IgG and IgM. As a control group, sera from 161 healthy blood donors were chosen. The number of tick bites in this group was unknown.

Serology

Indirect microimmunofluorescence assay (MIF). Antigen prepared from Vero cell-grown isolates of R. helvetica from Ixodes ricinus ticks was harvested, pelleted (10,000 $\times g$ for 10 min), resuspended in 10 ml of 0.01 *M* phosphate-buffered saline (PBS, pH 7.3) and the stock suspension was frozen at -70°C. The antigen was applied by a micro-pipette to each well of microscope slides, air-dried, fixed in acetone for 15 min and incubated with serial dilutions of serum as previously described [1,3]. A serum from a blood donor with no history of tick bite was used as negative control. As a positive control, prepared on a separate slide, a serum from a patient with proven Mediterranean spotted fever, with an end-point IgG titre of 1:160 confirmed at the Swedish Institute for Infectious Disease Control, was used. Immunoglobulin G (IgG) antibodies were detected by fluorescein isothiocyanate-conjugated (FITC) γ-chain-specific polyclonal rabbit anti-human IgG (Ref: F0202; Dako, Denmark). Sera were screened in dilutions at 1:20 and immunoreactive sera were tested in 2-fold serial dilutions from 1:40 to maximum 1:160 depending on the reactivity, to determine titres of IgG. Endpoints (titres) were defined as the highest serum dilution showing bright green fluorescence to the rickettsiae. Titres at or above 1/80 for IgG were considered positive.

Western blot. 100 µl of purified intact whole-cell R. helvetica antigen was dissolved in 200 µl Laemmli solution (Bio Rad) (62.5 mM Tris-HCl (pH 6.8), 25% glycerol, 2% SDS, 0.01% Bromophenol Blue, 5% β-mercaptoethanol). SDS-PAGE of the antigen was performed with a Criterion Tris-HCl 4–15% gradient separating gel and run for 55 min at 200V, 100 mA in a Criterion Cell (Bio Rad). After electrophoresis, antigen was transferred onto PVDF membrane in a Criterion Blotter apparatus at 10 V overnight and non-specific binding sites were blocked for 1 h with 5% non-fat dry milk in TBS followed by washing. The membrane was cut into strips that were overlaid with sera diluted 1/40 (the positive control was diluted 1/500) in 5% non-fat milk-TTBS, and incubated for 2 h. After washes, the strips were incubated with HRP-conjugated goat anti-human IgG (BioRad, CatNo 172-1050) diluted 1/1000 in 5% non-fat dry milk-TTBS and after additional washes detected using the HRP Conjugate Substrate Kit according to the manufacturer's instructions (BioRad). For the positive control goat anti-rabbit HRP-conjugated IgG was used (BioRad, CatNo 170-6515). A hyperimmune serum from rabbit immunized with R. helvetica isolated from tick, prepared at the Department of Immunology, Huddinge University Hospital, Stockholm, was used as a positive control. The secondary antibody alone served as an assay negative control.

Results

MIF

Antibodies to R. helvetica were in the tick-exposed groups detected in 9 (3.8%) of the 236 serum samples when a titre of 1/80 was chosen as cut-off. 6/137 (4.4%) were seropositive for R. helvetica in the Borrelia positive group, and 3/99 (3.0%) in the tick-exposed but Borrelia negative group. Since the study had a retrospective design, repeated samples that allowed identification of seroconversion were not available. Among blood donors 1/161 (0.6%) were seropositive for R. helvetica.

Western blot assay

An antibody-specific response to the high-molecular mass-specific protein antigens in the 110–150 kDa region was demonstrated in the tested seropositive sample (Figure 1). The secondary antibody alone, used as negative control, did not bind to any proteins.

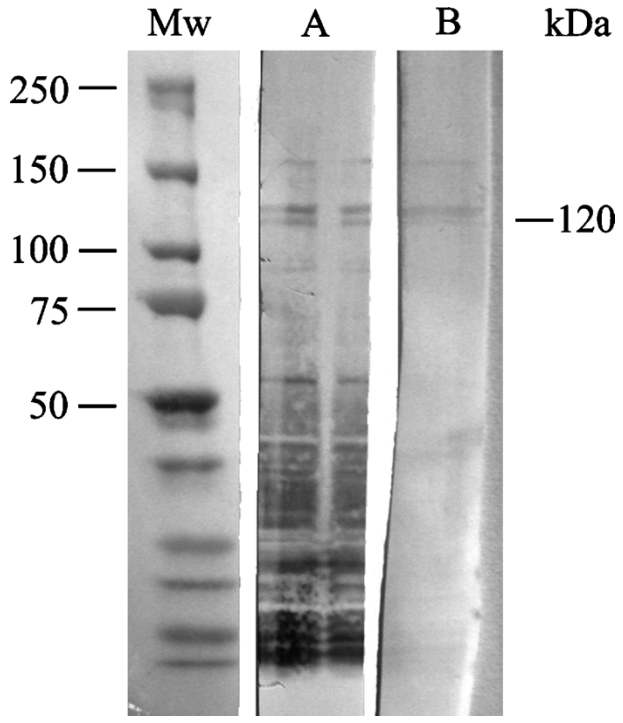

Figure 1. Western blot analysis of IgG antibodies to R. helvetica. Lane A demonstrates the lipopolysaccharide ladders and specific proteins reacting with a polyclonal rabbit antiserum. Lane B shows reaction against specific proteins in the 110–150-kDa region in a sample from 1 of the seropositive patients. No antibodies against LPS were demonstrated.

Discussion

In this study, when choosing 1/80 as a cut-off titre, IgG antibodies to R. helvetica were found in serum from 3.0–4.4% of subjects of the tick-exposed groups compared to 0.6% among healthy blood donors. The difference between the groups is statistically significant ($\chi^2 = 3.97$, df = 1, $p = 0.046$).

Previous studies from Sweden have by PCR demonstrated the presence of R. helvetica in both I. ricinus ticks and humans [4,6–8]. In Sweden, I. ricinus is the only vector of importance transmitting human pathogens. To date, I. ricinus, which is widely prevalent in Sweden and parts of Europe, has been demonstrated to harbour 3 rickettsial species, i.e. R. helvetica; secondly, a recently characterized spotted fever rickettsia reported from Slovakia and thirdly, R. monacensis isolated from I. ricinus in Germany [15,16]. R. helvetica is, to date, the only rickettsia isolated from ticks in Sweden. Hence, even if cross-reactions occur, seroconversion to Rickettsia after tick exposure in Sweden should mainly reflect antibodies to R. helvetica.

In the sample of 1 seroreactive subject, Rickettsia-specific protein antibodies were demonstrated by Western blot analysis. A pattern typical of antibodies to protein antigens of SFG rickettsiae, but no antibodies to lipopolysaccharide, was found. Notably in African tick bite fever, antibodies against LPS were detected in less than two-thirds of the patients [17].

In previous studies, ticks from different areas in central parts of Sweden were found, by PCR, to be positive for R. helvetica between 1.7 and 20% [18]. In a recent study from Denmark, R. helvetica was by PCR found in Ixodes ricinus ticks at less than 2% compared to Borrelia at 11% and A. phagocytophilum at 23.6% [19]. In a prospective serological study of Swedish recruits, who lived and trained in the eastern coastal areas, 8.9% showed seroconversion to R. helvetica and were also positive on re-examination with R. rickettsii as antigen [9]. A serosurvey in France found 9.2% positive to R. helvetica in a high-risk group of forest workers [2] and in Denmark 12.5% of serum samples of patients positive for Lyme borreliosis also had significant antibody titres to R. helvetica [10]. Our findings reflect the immune response to Rickettsia spp. in the central parts of Sweden, with a lower prevalence of Lyme borreliosis than the high-prevalent areas at the eastern and southern parts of Sweden. Sera from these areas should also be investigated to obtain a more conclusive picture of the prevalence of rickettsial antibodies among the Swedish population.

Three patients showed rickettsial antibodies and were tested negative for borreliosis. They had all reported arthralgia, myalgia and a general feeling of sickness. Concerning the blood donors, including the seropositive sample, we have no information about eventual tick exposition or symptoms, but the group represents an overall healthy part of the population.

The majority of patients with a confirmed infection with R. helvetica are reported to have a clinical presentation with mild non-specific symptoms but also a more severe septicaemic clinical picture has been demonstrated (unpublished observations). It is known from other countries that fever of unknown origin (FUO) could represent infections with R. helvetica between 1.7 and 11% [12,13], which means that the clinician also has to consider this agent in the assessment of febrile illnesses in Sweden. Both our study and the results from Denmark show that coinfection with rickettsiae occurs in some patients with acquired borreliosis, a possible coincidence that has to be taken into consideration when judging the clinical picture and making a choice of adequate treatment.

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